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Laser speckle imaging of intra organ drug distribution

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Abstract: Laminar flow in arteries causes streaming and uneven distribution of infused agents within the organ. This may lead to misinterpretation of experimental results and affect treatment outcomes. We monitor dynamical changes of superficial cortical blood flow in the rat kidney following different routes of administration of the vasoconstrictor angiotensin II. Our analysis reveals the appearance of large scale oscillations of the blood flow caused by inhomogeneous intra organ drug distribution.

OCIS codes: (120.6150) Speckle imaging; (170.1470) Blood or tissue constituent monitoring; (170.5380) Physiology.

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1. Introduction

One of the problems with intra-arterial infusion of drugs is that the infused solution may stream preferentially to certain branches of the arterial tree and therefore not be distributed uniformly in the tissue. This issue is of clinical importance with regard to drug delivery to target organs [1–4]. This can also lead to variability in experimental results and to possible misinterpretation of the data. Although the need to ensure adequate distribution of an infused drug to the kidney has been recognized [5–7] the visualization of local blood flow changes was not possible.

Recently, our group initiated the use of laser speckle flowmetry (LSF) to detect temporal and spatial flow changes on the kidney surface of anesthetized rats [8, 11]. The LSF measurements were based on the estimation of blood flow in efferent arterioles and their first-order branches (star vessels). The analysis revealed synchronous patterns of blood flow oscillations between groups of nephrons. Scully et al. [12], also using LSF on the surface of the rat kidney, analyzed oscillatory components in the blood perfusion during control conditions, enhanced autoregulation (L-NAME) and inhibited vasomotion (rho-kinase inhibitor Y-27632).

In this paper we demonstrate the use of laser speckle flowmetry to monitor the effect of intra organ drug distribution on superficial cortical blood flow in the kidney. Since laser speckle microscopy provides full-field real-time imaging of superficial blood flow we address the following questions: (i) How does homogeneity of drug distribution depend on different types of injections? (ii) What are the effects of uneven drug distribution? and (iii) How are local flow changes correlated to integral changes of the renal blood flow?

2. Methods

All experiments were performed on male Sprague-Dawley rats, body weight 270-330 g, purchased from Taconic (Lille Skensved, Denmark). All experimental protocols were approved by the Danish National Animal Experiments Inspectorate and were conducted in accordance with guidelines of the American Physiological Society.

Animal preparation was described in details in Ref. [8]. Here we give brief outlines. Rats were anesthetized by sevoflurane with the final concentration of 2-2.5% to maintain sufficient anesthesia. Mean arterial pressure was measured with Statham P23-dB pressure transducer (Gould, Oxnard, CA). Two PE-10 catheters in the right jugular vein allowed for continuous systemic infusions. After tracheotomy, the rat was connected to mechanical animal ventilator at a frequency of 60 breaths/min. A constant body temperature was maintained at 37 °C. The muscle relaxant cisatracurium (Nimbex, 0.85 mg/ml) was administred as 0.5 ml bolus followed by continuous infusion of 20 μl/min. For intra renal infusions a tapered and curved PE-10 catheter was introduced through the left iliac artery and advanced through the abdominal aorta and into the left renal artery. Ultrasonic flow probe (1PRB; Transonic T 420) was placed around the left renal artery to measure renal blood flow.

To eliminate motion artifacts caused by animal breathing the kidney was placed in a fixed plastic cup. To evaluate other possible motion artifacts we placed two hairs from the animal on the renal surface crossing each other. The motion artifacts were less than 50 μm that was insignificantly small in comparison with the region of interest. We used MoorFLPI setup (Moor Instruments, Millwey, Axminster, UK) to monitor blood flow on the kidney surface.

We performed three series of experiments: I) Rats (n=2) received systemic (intravenous) infusion of angiotensin II (AngII) dissolved in saline to the final concentration of $4ng/10$ μl at a rate of 20 μl/min into the right jugular vein; II) Rats (n=10) received continuous intrarenal infusion of AngII at the same concentration directly into the renal artery to minimize systemic effects; and III) Rats (n=5) received intrarenal bolus injections of AngII at two doses (1 ng and 2 ng) with 10 min interval between the injections into the renal artery. Before the infusions, a 20 min control period were performed. While recording superficial cortical blood flow changes

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with laser speckle microscopy, we monitored the arterial pressure with a pressure transducer (Statham P23-dB, Gould, Oxnard, CA), and total renal blood flow by an ultrasonic flow probe placed around the renal artery (1PRB; Transonic T 420).

Recorded raw data were processed using temporal laser speckle contrast analysis. Blood flow index, BFI, was calculated as in Ref. [13] :

$$BFI = \bar{I}^2/\sigma^2. \tag{1}$$

Here $\bar{I}$ and σ are the mean value and the standard deviation calculated over 25 frames for each pixel. BFI is proportional to the mean velocity of scattering particles (erythrocytes).

AngII is a well known vasoconstrictor that reduces superficial cortical blood flow as detected by LSF, as well as total renal blood flow as detected by ultrasonic flow probe on the renal artery [8]. To visualize changes in superficial cortical blood flow before and after administration of AngII, we mapped the pixel-by-pixel differences in the LSF images. For each pixel we calculated the ratio:

$$L(x,y) = (\bar{L}_{\text{control}}(x,y) - \bar{L}_{\text{AngII}}(x,y))/\bar{L}_{\text{control}}(x,y). \tag{2}$$

Here $\bar{L}_{\text{control}}$ and $\bar{L}_{\text{AngII}}$ are the mean LSF values over the time of control recordings and AngII effect, respectively, at each pixel (x,y) of the image. The time of AngII effect is defined as the interval between the maximal drop of blood flow in the renal artery after the infusion/injection and the end of the continuous infusion (or the increase of the renal blood flow once the bolus injection is over). The length of the control period was adjusted to match the period during which the AngII effect was assessed.

To visualize dynamical changes of LSF measurements we introduced Fourier mapping. For this, we calculated relative changes of Fourier power spectrum at each pixel of the image:

$$F(x,y) = \bar{F}_{\text{AngII}}(x,y)/\bar{F}_{\text{control}}(x,y), \tag{3}$$

where $\bar{F}_{\text{control}}$ and $\bar{F}_{\text{AngII}}$ are the mean Fourier power within the band of $f \in [0.0015; 0.015]$ Hz for control and AngII effect recordings, respectively. The upper frequency limit is chosen to exclude the peak at [0.02;0.03] Hz corresponding to the tubular glomerular feedback (TGF) oscillations [9].

3. Results

Spatial heterogeneity. First, we focus on characterization of the spatial heterogeneity of AngII effect. To visualize this effect we use a difference map (2). Figure 1 shows results for all groups of rats. Systemic infusion (group I) causes a uniform effect of AngII over the surface of the kidney (top panel). On the contrary, intrarenal infusion results in strong inhomogeneity of the drug effect in 9 out of 10 rats. This is manifested in the appearance of one to two well pronounced regions affected by the drug (red colored regions in Fig. 1, middle panel). Their relative area varies from 5-65% of the surface of the kidney. Rapid bolus injections also lead to inhomogeneous responses. Moreover, the affected area increases as the AngII dose increases (bottom panel). Results over all experimental groups are summarized in Fig. 2. One can see how much of the kidney surface is affected by AngII: intrarenal infusions/injections of AngII results in localized effects, while systemic infusion affects the whole kidney.

Temporal patterns. Second, we evaluate temporal changes of blood flow on the surface of the kidney caused by uneven drug distribution. Analysis shows alternating regions on the kidney surface affected by the drug (Fig. 3). Difference map for the rat II.9, for instance, demonstrates how blood flow redistributes between regions of interest marked as R1 and R2. Notice that the oscillations in the affected regions show anti-phase behavior, Fig. 3 (left bottom panel). We

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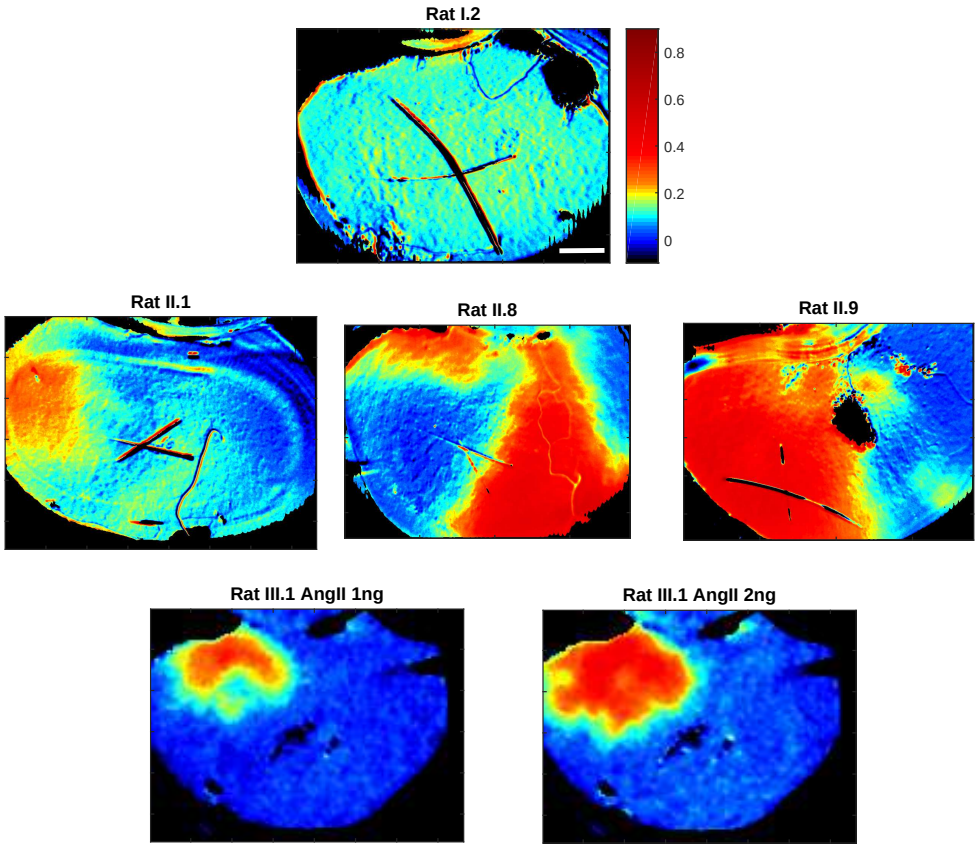

Fig. 1. Difference mapping of the AngII effect. L(x,y) values are color-coded (see colorbar). Top panel: Systemic infusion of AngII uniformly affects superficial renal cortical blood flow. Horizontal bar corresponds to 2.5 mm. Middle panel: Continuous intrarenal infusion directly into the renal artery. Different patterns of inhomogeneity are observed. Bottom panel: Bolus injections directly into the renal artery at two different doses. There is strong inhomogeneity with increasing area of the effect as concentration of AngII increases.

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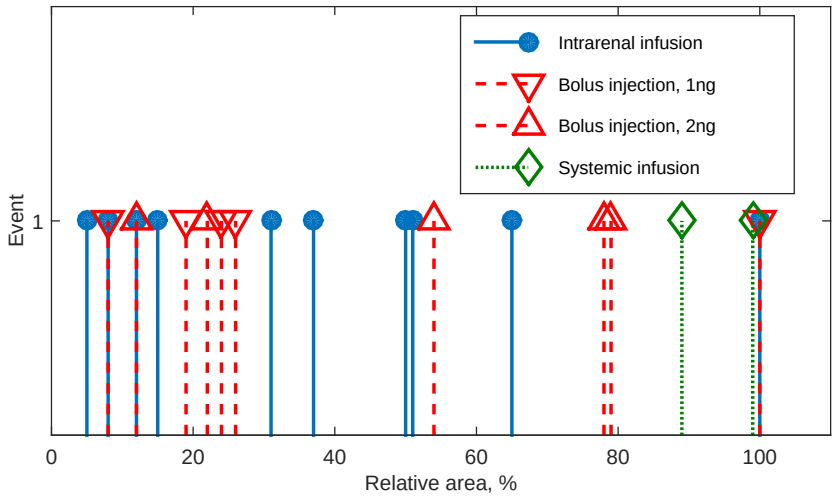

Fig. 2. Relative area of the regions affected by AngII for all groups of rats. One can see that most of the intrarenal infusions result in smaller areas being affected, and therefore in greater spatial inhomogeneity.

suggest the following explanation: (i) Laminar flow does not provide effective mixing of the drug with blood; (ii) As a result, some branches of the intrarenal vascular tree receive more AngII than others; (iii) The affected branches constrict and local blood flow is reduced, and (iv) This, in turn, redirects blood flow, and thus the delivery of AngII to the nearest upstream branches that will constrict while the previously constricted branches will dilate. The pattern repeats during the infusion period, and results in rhythmic constrictions and dilations of the vessels, and as a consequence, the appearance of large scale oscillations of blood flow on the surface of the kidney.

To study this effect in details we perform Fourier mapping (3) for each rat in the groups I and II in the frequency range [0.0015;0.015] Hz of very slow oscillations. This frequency range is chosen for two reasons: (i) AngII-induced constriction, flow redistribution, and subsequent dilation are slow processes and (ii) to exclude the TGF frequency from the analysis. The blood flow of the individual nephrons oscillates at a frequency in the range [0.02; 0.035] Hz, and AngII enhances these TGF mediated oscillations in nephron blood flow ([10]. These oscillations would contribute to the Fourier power, but are unrelated to the effects due to the uneven drug distribution. Figure 3 (right bottom panel) shows significant increase of the amplitude of the very slow oscillations in two localized regions, probably corresponding to two larger vascular branches affected by AngII. Table 1 summarizes the results on Fourier maps for all rats of the groups with systemic (I) and intrarenal (II) infusions. We define three threshold values for amplification in the range of very slow frequencies and calculate the relative area of the surface of the kidney with a certain degree of Fourier power amplification. A significant increase in the power of very slow oscillations is observed in most of rats following AngII infusion into the renal artery (group II).

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Table 1. Relative area (%) affected by AngII. Calculations are performed on the basis of amplification of the very slow frequency range in Fourier spectra.

Amplification \ Rats	I.1	I.2	II.1	II.2	II.3	II.4	II.5	II.6	II.7	II.8	II.9	II.10
< 1.2	80	97	52	67	27	71	62	69	73	60	20	34
[1.2; 5.0]	15	16	26	22	54	29	26	22	19	24	29	38
> 5.0	5	5	22	11	19	0	12	9	8	16	51	28

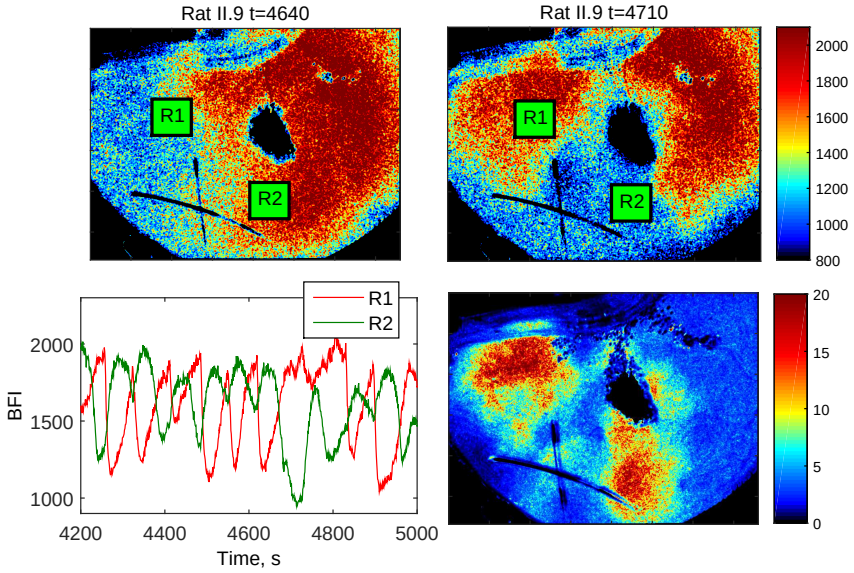

Fig. 3. Dynamical features of heterogeneity for intrarental infusions of AngII. Top panel: Snapshots of blood flow images at different moments of time, BFI index is color-coded according to the scale on the right side of the panel. Bottom panel, left: The average time series recorded from the regions R1 and R2. Bottom panel, right: Fourier relation map for the frequency band [0.0015; 0.015] Hz. Color-coded map shows $F(x,y)$ values. There are two regions with well-pronounced very slow oscillations corresponding to alternating patterns in the top panel.

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Full-field vs. integral characteristics. Finally, we are interested in how conventional methods used for renal measurements reflect the observed effects. To study this question the dynamics extracted from full-field LSF data was compared to renal blood flow measurements recorded by ultrasonic probe. Figure 4 compares time series from affected and non-affected regions of LS with renal artery blood flow (top panels) and composition of Fourier spectra at control conditions and under the effect of the drug (bottom panels). LS data clearly show variability of local blood flow on the surface that depends on inhomogeneous drug distribution. This means that groups of nephrons operate at different conditions that change in time. Fourier power spectrum shows the appearance of new frequency components after the infusion. On the other hand, recordings from the probe on the renal artery show just decreasing blood flow: While dynamical patterns on the surface of the kidney changes in time, renal blood flow shows stable overall effect.

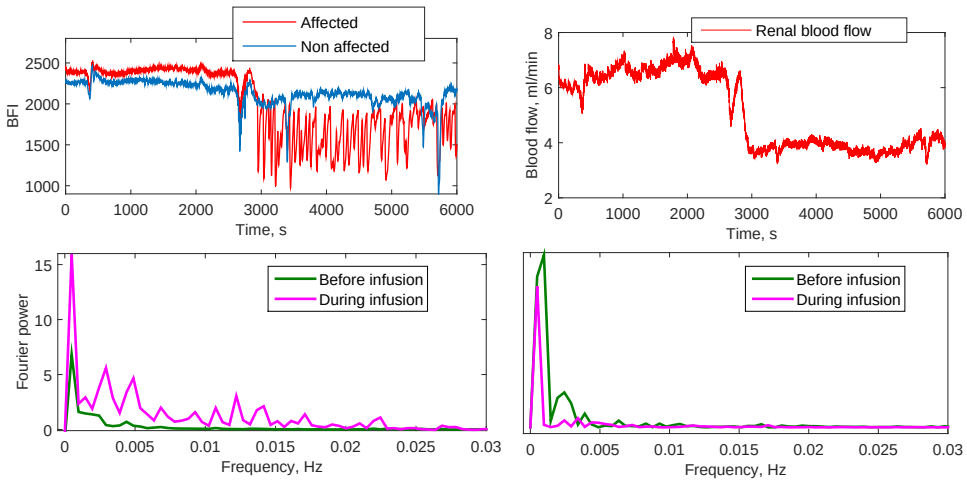

Fig. 4. Comparison of blood flow dynamics recorded by full-field LSF technique (left) and integral blood flow by ultrasonic probe on the renal artery (right). Top panel: Time series from blood flow images recorded from the region of the kidney surface affected (red) and not affected (blue) by AngII show high variability compared to the recordings of total renal blood flow (right panel). Bottom panel: Fourier spectra averaged over the kidney surface after (magenta) the infusion of AngII contain new low frequency components in comparison with Fourier spectra obtained before (green) infusion AngII, as well as from the renal blood flow recordings. One can see that total renal blood flow do not reflect the complex dynamics of local blood flow in the kidney.

4. Conclusion

Our results are in agreement with the data obtained by Parekh [5] who showed that intrarenal infusion of dye into the rat kidney results in extremely uneven distribution of coloration. We extended Parekh's studies to the direct monitoring of the inhomogeneous effects of intra arterial AngII infusions on local blood flow by means of laser speckle flowmetry technique, and evaluated the appearance of spatial and temporal patterns.

Laser speckle flowmetry analysis revealed that intrarenally infused drug carried away with the laminar blood flow in the renal artery to certain portions of the kidney led to strong localization of the drug effect in the form of inhomogeneous distribution of affected regions on the surface of the kidney. Localized regions alternated in time producing very slow oscillatory dy-

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namics on the surface of the kidney. As the result, it seems that integral characteristics, such as total renal blood flow, provide overall information while miss significant spatial and temporal aspects induced by inhomogeneous drug distribution. Full-field monitoring of the system can ensure better understanding of the functional state of the system.

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